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Influence of Auxin and Gibberellin on in Vivo Protein Synthesis during Early Pea Fruit Growth[1]

Rika van Huizen, Jocelyn A. Ozga*, and Dennis M. Reinecke

Department of Agricultural, Food, and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada, T6G 2P5

Developing pea fruits (*Pisum sativum* L.) offer a unique opportunity to study growth and development in a tissue that is responsive to both gibberellins (GAs) and auxin (4-chloroindole-3-acetic acid[4-Cl-IAA]). To begin a molecular analysis of the interaction of GAs and auxins in pea fruit development, in vivo labeling with [^{35}S]methionine coupled with two-dimensional gel electrophoresis were used to characterize de novo synthesis of proteins during gibberellic acid (GA_3)-, 4-Cl-indoleacetic acid-, and seed-induced pea pericarp growth. The most significant and reproducible polypeptide changes were observed between molecular weights of 20 and 60. Comparing about 250 de novo synthesized proteins revealed that seed removal changed the pattern substantially. We identified one class of polypeptides that was uniquely seed induced and five classes that were affected by hormone treatment. The latter included 4-Cl-IAA-induced, GA_3-induced, GA_3- and 4-Cl-IAA-induced, 4-Cl-IAA-repressed, and GA_3- and 4-Cl-IAA-repressed polypeptides. Similar patterns of protein expression were associated with both hormone treatments; however, changes unique to GA_3 or 4-Cl-IAA treatment also indicate that the effects of GA_3 and 4-Cl-IAA on this process are not equivalent. In general, application of 4-Cl-IAA plus GA_3 replaced the seed effects on pericarp protein synthesis, supporting our hypothesis that both hormones are involved in pea pericarp development.

The development of seeds and the surrounding ovary (fruit) is closely integrated, but little is known about the biochemical and molecular interplay between them. In pea (*Pisum sativum* L.) normal pericarp growth requires the presence of seeds (Eeuwens and Schwabe, 1975). Removal of the seeds 2 to 3 DAA results in the slowing of pericarp growth and subsequent abscission. The effect of seeds on fruit development, a process that involves cell division, cell enlargement, and cell differentiation, is assumed to involve plant hormones (Eeuwens and Schwabe, 1975; Gillaspy et al., 1993). Developing pea seeds contain GAs (biologically active GA_1 and GA_3; Garcia-Martinez et al., 1991) and auxins (4-Cl-IAA and IAA; Marumo et al., 1968; Ozga et al., 1993), and the effects of seeds on pericarp growth can be replaced by the application of GAs (Eeuwens and Schwabe, 1975) or 4-Cl-IAA (Reinecke et al., 1995).

It has been assumed that GAs biosynthesized by seeds are transported to the pericarp and regulate its growth. However, an alternative hypothesis that seeds may promote pericarp growth by maintaining its GA biosynthesis has been proposed (Sponsel, 1982). Results obtained using a split-pericarp system suggest that upon seed removal a key step in the GA biosynthesis pathway is inhibited (conversion of GA_{19} to GA_{20}; Ozga et al., 1992). The auxin 4-Cl-IAA can substitute for the seeds in the stimulation of pericarp growth and the conversion of GA_{19} to GA_{20} (van Huizen et al., 1995). Experiments in which the GA biosynthesis inhibitor paclobutrazol was used suggested that a portion of 4-Cl-IAA-stimulated growth is through its effect on GA biosynthesis (Brenner and Ozga, 1991). We have developed a working hypothesis that envisions two roles for auxin in controlling fruit growth: (a) the export of 4-Cl-IAA from the seeds to the pericarp, where it stimulates GA biosynthesis; and (b) a direct auxin effect of 4-Cl-IAA on pericarp growth. To test this hypothesis further and to broaden our understanding of hormone regulation of fruit development, critical analysis and timing of the molecular processes regulated by these hormonal signals are required.

Most of the molecular studies on the mechanism(s) of auxin and GA action have focused on auxin- or GA-induced cell elongation in vegetative tissues (coleoptiles, stems, hypocotyls, and epicotyls; Theologis, 1986; Chory et al., 1987). Auxin- and GA-mediated cell elongation is associated with changes in the expression of specific gene products (Theologis, 1986; Chory et al., 1987; Shi et al., 1992; Phillips and Huttly, 1994; Hagen, 1995). Several of these auxin- and GA-responsive genes have been characterized, and the functions of the proteins they encode have been proposed (Abel et al., 1994; Phillips and Huttly, 1994).

Investigations of fruit development using molecular genetic techniques have concentrated mainly on fruit ripening (Gray et al., 1992), although changes in polypeptide patterns (Veluthambi and Poovaiah, 1984) and expression of several genes have been reported during early fruit development in tomato, kiwifruit, strawberry, and pea (Narita and Gruissem, 1989; Reddy and Poovaiah, 1990; Reddy et al., 1990; Salts et al., 1991; Ledger and Gardner, 1994; Perez-Amador et al., 1995).

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* Corresponding author; e-mail jozga@afns.ualberta.ca; fax 1–403–492–4265.

Abbreviations: 4-Cl-IAA, 4-chloroindole-3-acetic acid; DAA, days after anthesis; OD, optical density; SP, split pericarp with seeds; SPNS, split pericarp no seeds.

Molecular studies investigating the regulatory role of auxin and GAs during early fruit development have focused on either auxin- or GA-regulated developmental processes (Veluthambi and Poovaiah, 1984; Reddy and Poovaiah, 1990; Reddy et al., 1990; Granell et al., 1992). Little is known about the relative roles of auxin and GA or their interaction in young, developing fruit. Our split-pod (pericarp) pea system offers the unique features of studying growth and development in a fruit in which the seeds are accessible to manipulation and the pericarp is responsive to both GA and auxin (4-Cl-IAA) in planta (Ozga and Reinecke, 1994; Reinecke et al., 1995). To begin a molecular analysis of the interaction of GAs and auxins during pea fruit development we have utilized in vivo protein labeling with [^{35}S]Met coupled with two-dimensional PAGE to obtain a profile of hormonal and seed-induced polypeptide changes during early fruit development. We report here the protein expression patterns associated with 4-Cl-IAA-, GA_3-, and seed-induced pea pericarp growth.

MATERIALS AND METHODS

Plants of *Pisum sativum* L. line I_3 (Alaska-type) were grown as previously described (van Huizen et al., 1995). One fruit per plant (at the third to fifth flowering node) was treated, and subsequent flowers were removed as they developed. Terminal apical meristems of plants were intact, and the pericarp remained attached to the plant during the entire experiment. To remove the seeds a split-pericarp technique was used as described by Ozga et al. (1992). Pericarps of 2 DAA (15–20 mm) ovaries (pericarp plus seeds) were split down the dorsal suture, and the seeds either were not disturbed (SP treatment) or were removed immediately (SPNS treatment). Eight or 14 h later, the SP and SPNS pericarps were treated with 10 μCi of L-[^{35}S]Met (>1000 Ci/mmol; Amersham, Canada) in 10 μL of 0.1% Tween 80. Two hours prior to [^{35}S]Met application (14 h treatments only) deseeded pericarps were treated with GA_3 and/or 4-Cl-IAA (30 μL, 50 μM in 0.1% Tween 80) or 0.1% Tween 80 alone (SP and SPNS controls). In some cases paclobutrazol, an inhibitor of GA biosynthesis, was applied to the pericarp (30 μL, 50 μM in 0.1% Tween 80) immediately after deseeding. All treatments were applied to the inside surface (endocarp) of the pericarp. After a 4-h incubation period with L-[^{35}S]Met the pericarps (seeds were removed if present) were harvested 12 h (SP and SPNS) or 18 h (SP, SPNS, and hormone treatments) after pericarp splitting, frozen immediately in liquid N_2, and stored at −80°C until extracted. High humidity was maintained by enclosing the fruits in clear plastic bags throughout the duration of the experiment.

Extraction of Total Protein

Total proteins were extracted as follows. Frozen pericarps (two per sample) were homogenized to a fine powder in a mortar containing liquid N_2. When the temperature reached approximately 0°C, 0.5 mL of extraction buffer (50 mM Tris-HCl, pH 7.5, 2% SDS, 150 mM DTT, and 1 mM PMSF) was added. The extract was centrifuged at 10,000*g* for 10 min, and the clear supernatant was heated at 100°C for 3 min and cooled on ice. The proteins were precipitated by adding cold acetone (8× volume of supernatant) and kept at −20°C for at least 1 h. The acetone precipitates were pelleted by centrifugation at 10,000*g* for 10 min at 4°C. After the acetone was removed the pellet was air-dried and resuspended in buffer containing 9.5 M urea, 5 mM K_2CO_3, 4% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (Sigma), and 5% ampholytes (2 parts pH 4–5, 2 parts pH 5–7, and 1 part pH 3–10; Bio-Rad). The insoluble material was removed by centrifugation at 10,000*g* for 30 min. An aliquot containing 400,000 TCA-precipitable dpm was loaded onto each IEF gel; the total protein loaded per IEF gel was approximately 80 μg.

Two-Dimensional Gel Electrophoresis

Two-dimensional PAGE was carried out as described by Xin and Li (1993) with the following modification: The final combined concentration of ampholytes in the IEF gel solution was 5% (2 parts pH 4–5, 2 parts pH 5–7, and 1 part pH 3–10). Second-dimension electrophoresis was performed as described by Laemmli (1970) using 4% acrylamide for the stacking gel and 14% acrylamide for the running gel. Gels were run at a constant current of 25 mA/gel at 18°C for 3.5 h. After overnight fixation in methanol:acetic acid:H_2O (40:10:50 [v/v]) gels were infiltrated with 2,5-diphenyloxazole as described by Laskey and Mills (1975). Gels were dried onto Whatman 3 MM paper and exposed to pre-flashed Kodak X-Omat AR x-ray film at −80°C for approximately 10 d. All treatments were repeated a minimum of three times, and the reproducible changes were analyzed by scanning the film with an imaging densitometer (Bio-Rad). For integration of x-ray film images, Bio-Rad Molecular Analyst/PC Image Analysis software (version 1.3) was used. The area of each individual protein spot image was approximated best by enclosing it within an ellipse. The total digital signal or OD within an ellipse was determined, and a background OD value for each protein spot image was determined by selecting an area of the same size free from visible protein spots on the fluorograph image close to the protein spot to be integrated. This background OD value was subtracted from the total OD of the protein spot image to remove values contributed from background noise. The percentage of relative abundance of specific radiolabeled polypeptides was determined by comparing the OD of the specific protein with a standard protein with a labeling pattern that was consistent between treatments (the same standard protein was used to normalize all samples) using the following formula:

$$\text{Relative abundance (\%)} = \frac{\text{Total OD (specific protein)} - \text{background OD}}{\text{Total OD (standard protein)} - \text{background OD}} \times 100.$$

RESULTS

Growth (length) was similar in pericarps with and without seeds 8 h (2.3 ± 0.3 mm) or 12 h after pericarp splitting

(prior to hormone application; 3.5 ± 0.3 mm). However, during the 6-h hormone incubation period, pericarp growth differed significantly among treatments (F test; $P < 0.0001$). SPNS growth was significantly reduced compared with SP (LSD, $P = 0.5$; Fig. 1). GA_3 and 4-Cl-IAA significantly stimulated the growth of deseeded pericarps compared with the deseeded controls (SPNS; LSD, $P = 0.5$). The combination of GA_3 plus 4-Cl-IAA had additive effects on the growth of deseeded pericarps, which resulted in growth similar to that of pericarps with seeds. The addition of paclobutrazol had only a minor effect on the growth response to 4-Cl-IAA with or without GA_3.

Analysis of de novo protein synthesis revealed that the most significant and reproducible changes were observed in polypeptides with M_rs between 20 and 60 (Figs. 2 and 3). The polypeptide changes were grouped into six classes: 4-Cl-IAA induced, 4-Cl-IAA repressed, GA_3 induced, GA_3 and 4-Cl-IAA induced, GA_3 and 4-Cl-IAA repressed, and seed induced (Table I). The greatest difference in in vivo labeled polypeptide patterns occurred between pericarps with seeds and SPNS treatments (Figs. 2 and 3, A and B). Seed removal 8 h prior to [^{35}S]Met application resulted in the appearance of or an increase in 6 (3, 4, 7, 8, 9, and 11) labeled polypeptides and the disappearance or decrease in 9 (A–I) labeled polypeptides (Fig. 2). The pattern of labeled polypeptides in pericarps deseeded 14 h prior to [^{35}S]Met application was similar but not identical to the 8-h treatment pattern (Figs. 2 and 3, A and B). In pericarps deseeded 14 h prior to [^{35}S]Met application, polypeptide 9 had increased and 5 additional labeled polypeptides had

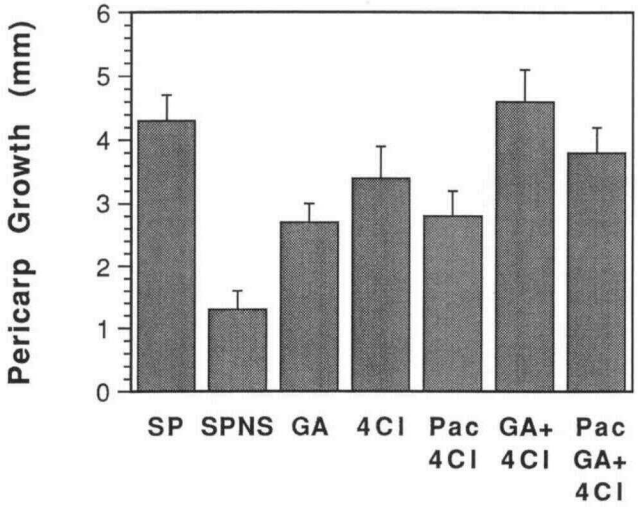

Figure 1. The effect of seeds (SP), seed removal (SPNS), and seed removal plus treatment with GA_3 (GA), 4-Cl-IAA (4 Cl), paclobutrazol plus 4-Cl-IAA (Pac 4 Cl), GA_3 plus 4-Cl-IAA (GA + 4 Cl), and paclobutrazol plus GA_3 plus 4-Cl-IAA (Pac GA + 4 Cl) on pea pericarp growth. Pericarps were deseeded 2 DAA and 12 h prior to GA_3 and/or 4-Cl-IAA (50 μM) or 0.1% Tween 80 application (SP and SPNS controls). Paclobutrazol was applied to the pericarp (50 μM) immediately after deseeding. Pericarp growth = final length (6 h after hormone treatment) minus initial length at the time of hormone application; data are means ± SE ($n = 6$).

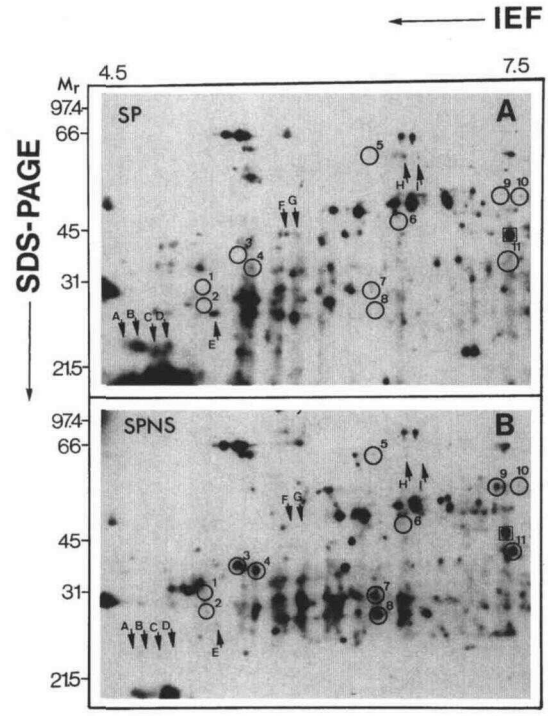

Figure 2. Fluorographs of [^{35}S]Met-labeled polypeptides from pericarps 2 DAA resolved by electrophoresis on two-dimensional gels. Pericarps were split (SP; A) or split and deseeded (SPNS; B) 8 h prior to [^{35}S]Met application. Letters (A–I) adjacent to arrows designate polypeptides enhanced by hormone and/or seed treatments (protein G is to the lower right of the arrow). Numbers (1–11) adjacent to circles designate polypeptides repressed by hormone and/or seed treatments. The standard protein used to normalize the abundance of specific radiolabeled polypeptides is enclosed within a square on each fluorograph. Each treatment was repeated at least three times, and representative fluorographs are shown.

Table I. *Summary of the major in vivo labeled polypeptide changes in pea pericarp 2 DAA as a result of GA_3, 4-Cl-IAA, or seed treatments*

Polypeptide Induction			Polypeptide Repression		
Inducer	ID[a]	M_r	Repressor	ID	M_r
4-Cl-IAA	A	24.7	4-Cl-IAA	5	62.8
	B	24.3		9	54.5
	C	23.7		10	53.6
	D	24.9			
GA_3 and 4-Cl-IAA	E	28.5	GA_3 and 4-Cl-IAA	1	32.3
				2	29.4
GA_3	F	45.0		3	38.4
	G	45.4		4	37.2
				6	47.2
Seed	H	63.8		7	30.8
	I	63.8		8	28.5
				11	39.7

[a] Polypeptide nomenclature.

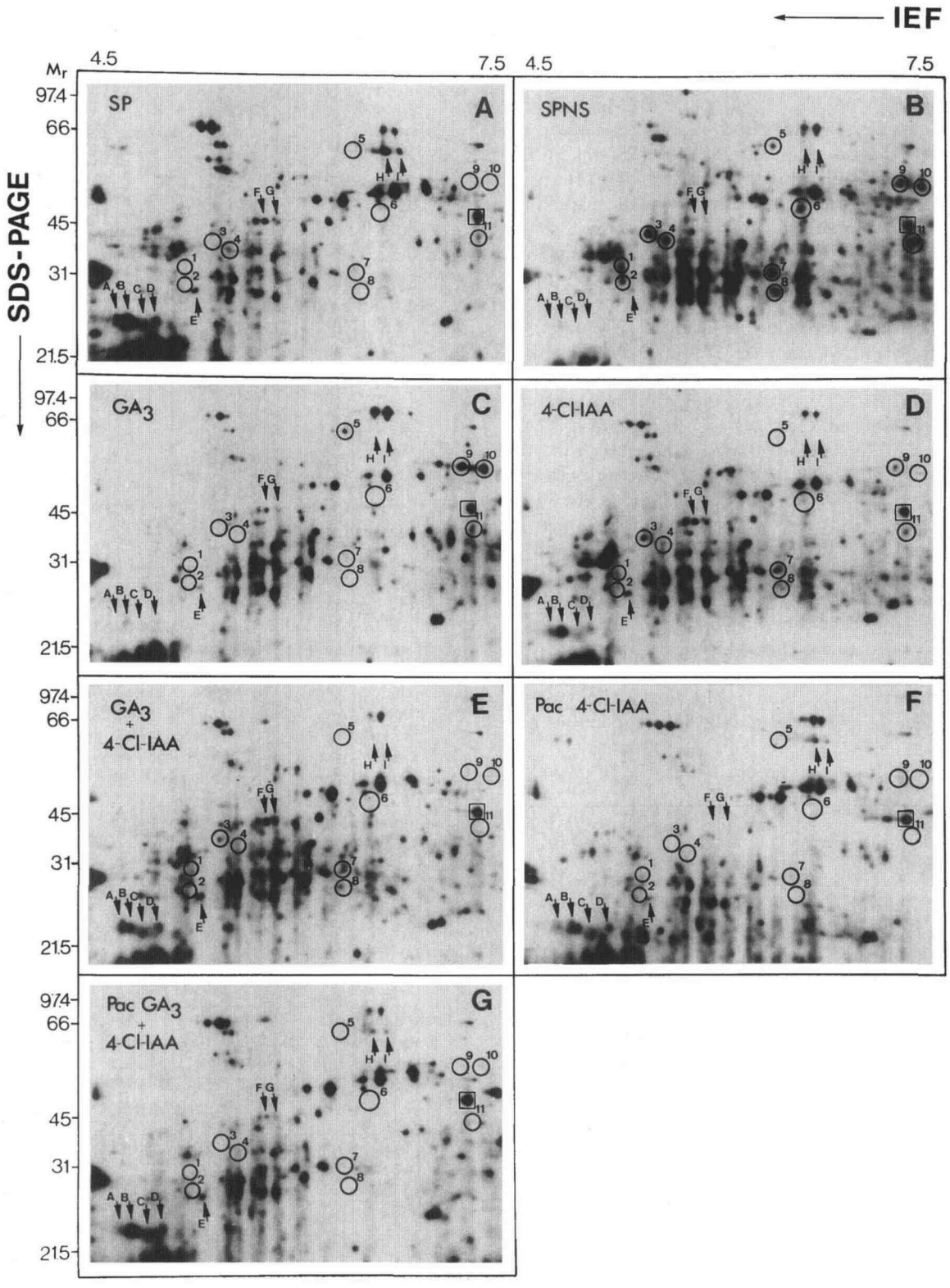

Figure 3. Fluorographs of [^{35}S]Met-labeled polypeptides from pericarps 2 DAA resolved by electrophoresis on two-dimensional gels. Pericarps were split (SP; A) or split and deseeded (B, C, D, E, F, and G) 14 h prior to [^{35}S]Met application. Two hours prior to [^{35}S]Met application pericarps were treated with 0.1% Tween 80 (controls: SP, A; and SPNS, B) or 50 μM solutions of GA_3 (C), 4-Cl-IAA (D), GA_3 plus 4-Cl-IAA (E), paclobutrazol plus 4-Cl-IAA (F), or paclobutrazol plus GA_3 plus 4-Cl-IAA (G). Polypeptide nomenclature and standard protein designations are the same as in Figure 2. Each treatment was repeated at least three times, and representative fluorographs are shown.

appeared or increased (1, 2, 5, 6, and 10; Fig. 3B) in comparison with pericarps deseeded 8 h prior to [^{35}S]Met application (Fig. 2).

Treatment of deseeded pericarps with 4-Cl-IAA or 4-Cl-IAA plus GA_3 inhibited accumulation of all 11 polypeptides (1–11) induced by seed removal to levels similar to those observed in pericarps with seeds (Fig. 3; abundance of selected polypeptides, Fig. 4, E and F). Application of GA_3 alone to deseeded pericarps suppressed only 8 of these 11 polypeptides (polypeptides 1, 2, 3, 4, 6, 7, 8, and 11). The net synthesis of polypeptides specifically inhibited by 4-Cl-IAA (5, 9, and 10; Fig. 4E) was 3 to 11 times higher in the deseeded (SPNS, 14-h treatment) and GA_3-treated deseeded pericarps than in pericarps treated with 4-Cl-IAA (Fig. 4E). Polypeptides 5, 9, and 10 also were in low abundance in pericarps with seeds (SP).

The abundance of four labeled polypeptides (A, B, C, and D) that exhibited less labeling following deseeding increased in deseeded pericarps when treated with 4-Cl-IAA, but not with GA_3 alone (Fig. 3). The levels of polypeptide B in 4-Cl-IAA-treated tissue were similar to those in pericarps with seeds, and approximately 6 to 10 times higher

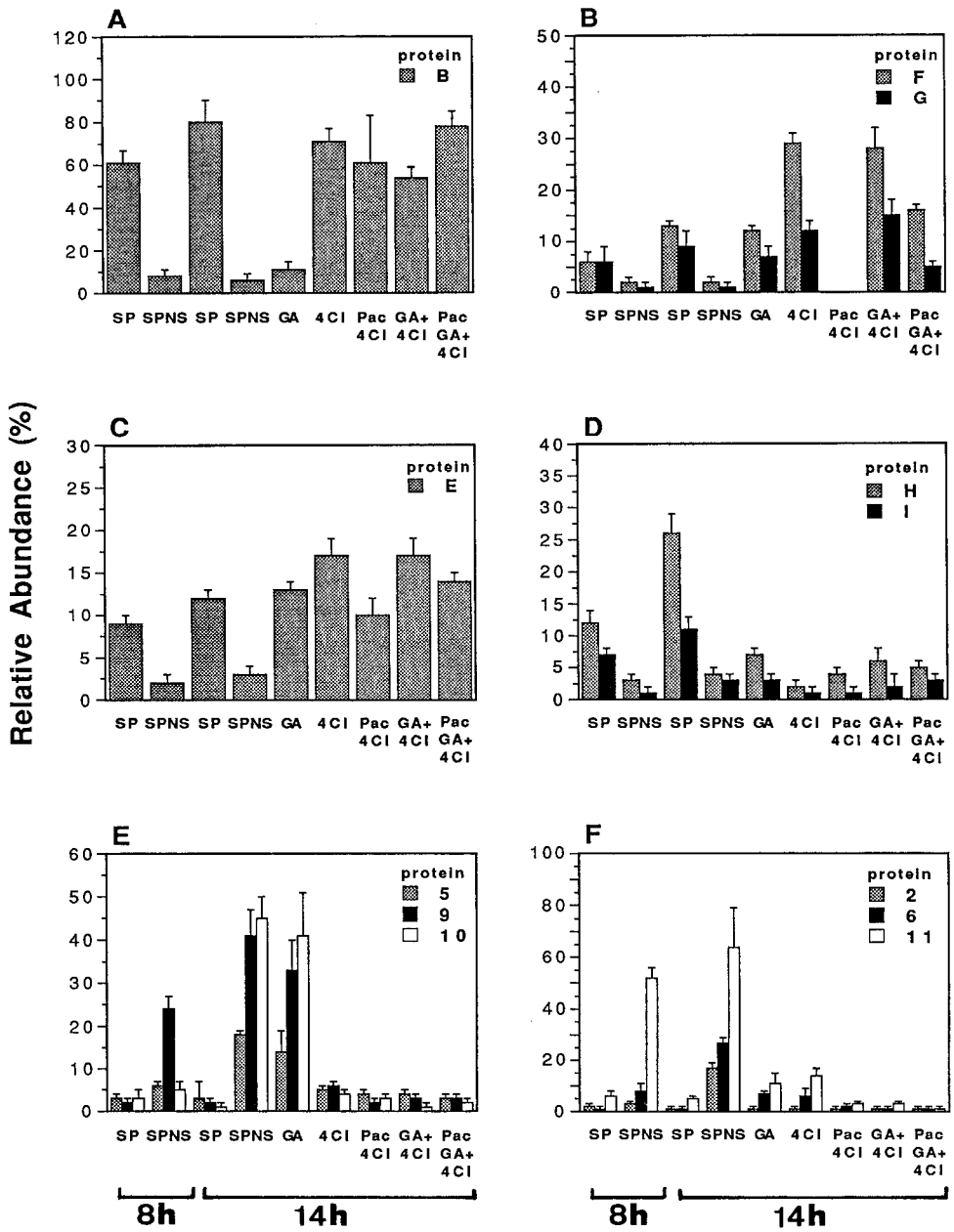

Figure 4. Relative abundance of six classes of [^{35}S]Met-labeled polypeptides in pea pericarp: 4-Cl-IAA induced (A), GA_3 induced (B), GA_3 plus 4-Cl-IAA induced (C), seed-specific induced (D), 4-Cl-IAA repressed (E), GA_3 plus 4-Cl-IAA repressed (F). Quantitation of fluorographs was accomplished as described in "Materials and Methods." The results represent the average of three replicates ± SE, with one exception (for Pac 4Cl treatment, n = 2).

than those in GA_3-treated deseeded pericarps and SPNS controls (Fig. 4A).

Because 4-Cl-IAA has the capacity to stimulate a key step in the GA biosynthesis pathway (GA_{19} to GA_{20}) paclobutrazol was applied to resolve the selective contribution of 4-Cl-IAA and GA_3 on protein synthesis in early pea fruit growth. Paclobutrazol inhibited the accumulation of polypeptides F and G in tissue treated with 4-Cl-IAA (Fig. 4B, Pac 4Cl), but not in pericarp treated with both 4-Cl-IAA and GA_3 (Fig. 4B, Pac GA_3 + 4-Cl). Polypeptides F and G were 6 to 15 times more abundant in the GA_3-, 4-Cl-IAA-, and GA_3 plus 4-Cl-IAA-treated deseeded pericarps than in the SPNS controls. Polypeptides F and G also were detected in pericarp with seeds (SP, 14-h treatment) at levels 6 and 9 times higher, respectively, than in deseeded pericarp (SPNS). These data suggest that polypeptides F and G are regulated by GAs.

One polypeptide (E) was induced in both GA_3- and 4-Cl-IAA-treated deseeded pericarps (Fig. 3). Its levels were approximately 4 to 6 times higher in the GA_3-, 4-Cl-IAA-, and GA_3 plus 4-Cl-IAA-treated deseeded pericarps than in the SPNS (Fig. 4C). Polypeptide E also was present in SP at a level similar to that found in hormone-treated pericarps.

Among the polypeptides analyzed, two were more abundant (H, 4–13 times; I, 4–11 times) in SP than in deseeded pericarps, regardless of the treatment (Fig. 3). Labeling of polypeptide H in the SP treatment doubled from the 8- to the 14-h treatment period (Fig. 4D).

Since the combination of 4-Cl-IAA plus GA_3 stimulates pericarp growth more than 4-Cl-IAA or GA_3 alone (Ozga and Reinecke, 1994), we compared protein profiles of 4-Cl-IAA plus GA_3-, 4-Cl-IAA-, and GA_3-treated deseeded pericarps. 4-Cl-IAA plus GA_3 and 4-Cl-IAA alone were very similar in enhancing and suppressing the synthesis of proteins in pericarp tissue (Figs. 3, D and E, and 4, A, C, E, and F).

DISCUSSION

Our previous growth and metabolism studies showed that 4-Cl-IAA, an endogenous pea auxin, can stimulate pericarp growth and GA biosynthesis in the pericarp, specifically the conversion of GA_{19} to GA_{20}. In this study the effect of GA, auxin, and seeds on in vivo protein synthesis in pea pericarp was used to assess, at the protein level, the relative contribution of these hormones to pea fruit development and to evaluate the extent to which these hormones can mimic the seed in this process. Our data reveal that seed removal greatly modifies in vivo labeled protein patterns. As soon as 12 h after seed removal, six labeled polypeptides appeared or increased (deseeded 8 h prior to [^{35}S]Met application; Fig. 2). An additional five polypeptides appeared or increased 18 h after pericarp deseeding (deseeded 14 h prior to [^{35}S]Met application; Fig. 3). These pericarps are still viable after seed removal, as shown by their growth response to delayed hormonal application (GA_3 plus 4-Cl-IAA; van Huizen et al., 1995). During early pea fruit development, seed factors may normally suppress the synthesis of this class of polypeptides.

Hormonal effects on protein synthesis in deseeded pericarp were detected within 6 h of hormone application. Labeling of all 11 polypeptides specific to deseeded pericarp was inhibited to levels similar to that in pericarps with seeds by 4-Cl-IAA application (Figs. 3 and 4, E and F). Application of GA_3 to deseeded pericarps suppressed only 8 of these 11 polypeptides (polypeptides 1, 2, 3, 4, 6, 7, 8, and 11). Since growth of the GA_3- and 4-Cl-IAA-treated deseeded pericarps were similar (Fig. 1), polypeptides 5, 9, and 10 appear to be auxin-repressed specifically and not repressed by GA_3 or indirectly by growth. Auxin- or GA-repressed polypeptide synthesis has been characterized in various plant systems (Theologis, 1986; Chory et al., 1987). Veluthambi and Poovaiah (1984) found that removal of the achenes, which inhibits strawberry fruit growth, resulted in the appearance of two polypeptides of 52 and 57 kD. Exogenous NAA, which induces growth in receptacles from which achenes have been removed, prevented the appearance of these two polypeptides. The authors suggested that these proteins may have an inhibitory role in strawberry fruit development. This also may be the case for polypeptides 1, 2, 3, 4, 6, 7, 8, and 11 observed in pea fruit. However, we have observed an additional class of proteins (polypeptides 5, 9, and 10) that appear to be repressed specifically by auxin but not indirectly by pericarp growth.

In pea fruit, endogenous 4-Cl-IAA may act as a seed signal, stimulating GA biosynthesis in pericarp tissue (Ozga et al., 1992; van Huizen et al., 1995). Paclobutrazol was applied to resolve the effects of 4-Cl-IAA and GA_3 on protein synthesis in early pea fruit growth. Two proteins (F and G; Fig. 4B) were identified as GA_3 induced because they were not detected in deseeded pericarps treated with paclobutrazol plus 4-Cl-IAA but were present when GA_3 was added. Since proteins F and G are also present in pericarps with seeds and deseeded pericarps treated with 4-Cl-IAA only (Fig. 4B), these data support the hypothesis that part of the 4-Cl-IAA effect on pericarp growth is through stimulation of GA biosynthesis.

4-Cl-IAA also increased labeling or appearance of four proteins (A–D; Fig. 3; Table I), which were of low abundance or not detected in deseeded pericarps treated with GA_3 alone. These four proteins also were present in pericarps with seeds; therefore, endogenous 4-Cl-IAA may induce synthesis of proteins involved in pericarp growth. Veluthambi and Poovaiah (1984) found that NAA applied to strawberry fruit with achenes removed stimulated fruit growth and the accumulation of three polypeptides. By simultaneously monitoring auxin- and GA-induced protein synthesis, we observed a class of proteins (polypeptides A–D) that appear to be specifically induced by auxin but not by GA_3-induced pericarp growth. These results support our hypothesis that 4-Cl-IAA also has a direct auxin-mediated effect on pericarp growth.

Two polypeptides (H and I; Fig. 4D) were abundant in the pericarps only when the seeds were present; neither GA_3 nor 4-Cl-IAA induced their synthesis. One possible explanation is that seed factors other than 4-Cl-IAA or GA_3

may be involved in regulating protein synthesis in pea pericarps. Alternatively, these proteins may be induced by 4-Cl-IAA and/or GA_3 but after a longer incubation time.

Pea fruit growth is a carefully regulated process in time and space, beginning with pollination and fertilization, followed by pericarp growth, and, finally, seed growth and maturation. GAs and auxins are natural constituents of pea fruit, which likely play a role in pea fruit growth and development. Within 12 h after deseeding, pericarp tissue responds to seed removal by synthesizing, inhibiting synthesis, or modifying specific proteins (Fig. 2). Application of 4-Cl-IAA and GA_3 to pericarps 12 h after deseeding reverses this process (Figs. 3 and 4). Similar patterns of protein expression were associated with both hormone treatments; however, polypeptide patterns unique to GA_3 or 4-Cl-IAA treatment also indicate that their effects on this process are not equivalent. In general, application of 4-Cl-IAA plus GA_3 replaced the seed effect on protein synthesis in the pericarp, supporting our hypothesis that both hormones are involved in pea pericarp development. The results presented are unique in that both GA_3 and 4-Cl-IAA effects on protein synthesis were monitored simultaneously in a tissue system that naturally contains 4-Cl-IAA and GA_3. These data suggest that at least part of seed regulation of in vivo protein synthesis in the pericarp tissue is directly through hormonal signals from the seeds, other seed factors, or events that modify hormonal levels in the pericarp. Using the pea split-pericarp growth system, we intend to examine further the relative roles of auxins and GAs on pericarp development at the molecular level.

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